FISH & RICHARDSON P.C.

Frederick P. Fish
1855-1930

W.K. Richardson
1859-1951

225 Franklin Street
Boston, Massachusetts
02110-2804

Telephone
617 542-5070

Facsimile
617 542-8906

Web Site
www.fr.com

November 8, 1999

Attorney Docket No.: **10861/011003**

Box Patent Application
Assistant Commissioner for Patents
Washington, DC 20231

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Presented for filing is a new continuation patent application of:

Applicant: DENISA D. WAGNER AND ROBERT C. JOHNSON
Title: METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS

The prior application is assigned of record to Center for Blood Research, Inc., a Massachusetts corporation, by virtue of an assignment submitted to the Patent and Trademark Office for recording on April 19, 1995 at 7441/0041.

Enclosed are the following papers, including those required to receive a filing date under 37 CFR §1.53(b):

	Pages
Specification	26
Claims	5
Abstract	1
Declaration	1

Enclosures:

- Copy of Petition for Extension of Time.
- Copy of Declaration, which was filed in the parent application. Please enter these in this application.
- Preliminary amendment, 6 pages.
- Postcard.

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Date of Deposit November 8, 1999

I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as **"Express Mail Post Office To Addressee"** with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Joanne D. Boyle

Joanne D. Boyle

This application is a continuation (and claims the benefit of priority under 35 USC 120) of U.S. application serial no. 08/948,393, filed October 10, 1997. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

Basic filing fee	760.00
Total claims in excess of 20 times $18.00	72.00
Independent claims in excess of 3 times $78.00	156.00
Fee for multiple dependent claims	0.00
Total filing fee:	$ 988.00

A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at 617/542-5070.

Kindly acknowledge receipt of this application by returning the enclosed postcard.

Please send all correspondence to:

Louis Myers
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804

Respectfully submitted,

Louis Myers
Reg. No. 35,965
Enclosures

402775.B11

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Wagner et al. Serial No: Filed: For: METHODS FOR TREATING AND PREVENTING ATHEROSCLEROSIS Attorney Docket No.: 10861/011003	Group Art Unit: Examiner:

Assistant Commissioner for Patents
Washington, DC 20231

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November 8, 1999
Date of Signature and of Mail Deposit

By: Joanne D. Boyle

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination, please amend the above-identified application as follows:

In the specification:

Please replace the first sentence on page 1 of the specification, as amended in Applicants' amendment of October 8, 1997, as follows:

This application is a continuation of application Serial No. 08/948,393, filed October 8, 1997, which [This application] is a continuation of application Serial No. 08/377,798, filed January 24, 1995, which is a continuation-in-part of application Serial No. 08/253,663, filed June 3, 1994, now abandoned. The entire contents of the parent application are hereby expressly incorporated herein by reference.

In the claims:

Please cancel claims 1-38 and add new claims 39-63:

-- 39. A method for treating or preventing restenosis in a mammal to which a vessel-corrective technique selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery is administered, comprising:

performing a vessel-corrective technique selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery in a mammal; and

administering to said mammal, in conjunction with or after said vessel-corrective technique, an effective amount of an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, such that the restenosis occurring after said vessel-corrective technique is thereby treated or prevented.

40. A method for treating or preventing restenosis in a mammal, comprising:

providing an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, said agent being selected from the group consisting of an inhibitory protein, an inhibitory peptide, an inhibitory carbohydrate, an inhibitory glycoprotein, and a substance obtained from a snake venom or a plant extract; and

administering to a mammal an effective amount of said agent such that said P-selectin-ligand interaction is inhibited, wherein said agent is administered in conjunction with or after a vessel-corrective technique.

41. The method of claim 40, wherein said vessel-corrective technique is selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery.

42. The method of claim 40, wherein said agent comprises a soluble form of a P-selectin ligand or a fragment thereof.

43. The method of claim 42, wherein said P-selectin ligand is PSGL-1 or a fragment thereof.

44. The method of claim 40, wherein said agent comprises a chimeric construct between a P-selectin ligand or fragment thereof and another molecule.

45. The method of claim 44, wherein said chimeric construct comprises PSGL-1 or a fragment thereof.

46. The method of claim 40, wherein said inhibitory glycoprotein is a glycoprotein containing sialyl-Lewis x.

47. The method of claim 40, wherein said agent further inhibits an interaction between E-selectin and a ligand of E-selectin.

48. The method of claim 40, further comprising administering to said mammal a second agent which inhibits an interaction between E-selectin and a ligand of E-selectin, wherein said second agent is selected from the group consisting of an inhibitory protein, an inhibitory peptide, an inhibitory carbohydrate, an inhibitory glycoprotein and a substance obtained from a snake venom or a plant extract.

49. The method of claim 40, wherein said agent is administered in sequential exposures over a period of hours, days, weeks, months or years.

50. The method of claim 40, wherein said agent is administered in combination with other therapeutic agents.

51. A method for treating or inhibiting atherosclerosis in a mammal, comprising:

providing an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, said agent being selected from the group consisting of an inhibitory protein, an inhibitory peptide, an inhibitory carbohydrate, an inhibitory glycoprotein and a substance obtained from a snake venom or a plant extract; and

administering to a mammal an effective amount of said agent such that said P-selectin-ligand interaction is inhibited, wherein said agent is administered in conjunction with or after a vessel-corrective technique.

52. The method of claim 51, wherein said vessel-corrective technique is selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery.

53. The method of claim 51, wherein said agent comprises a soluble form of a P-selectin ligand or a fragment thereof.

54. The method of claim 53, wherein said P-selectin ligand is PSGL-1 or a fragment thereof.

55. The method of claim 51, wherein said agent comprises a chimeric construct between a P-selectin ligand or fragment thereof and another molecule.

56. The method of claim 55, wherein said chimeric construct comprises PSGL-1 or a fragment thereof.

57. The method of claim 51, wherein said agent further inhibits an interaction between E-selectin and a ligand of E-selectin.

58. The method of claim 51, further comprising administering to said mammal a second agent which inhibits an interaction between E-selectin and a ligand of E-selectin, wherein said second agent is selected from the group consisting of an inhibitory protein, an inhibitory peptide, an inhibitory carbohydrate, an inhibitory glycoprotein, and a substance obtained from a snake venom or a plant extract.

59. The method of claim 51, wherein said agent is administered in sequential exposures over a period of hours, days, weeks, months or years.

60. The method of claim 51, wherein said agent is administered in combination with other therapeutic agents.

61. A chimeric construct for inhibiting an interaction between P-selectin and a ligand of P-selectin, comprising a P-selectin ligand or a fragment thereof and another molecule.

62. The chimeric construct of claim 61, wherein said P-selectin ligand is PSGL-1 or a fragment thereof.

63. A method for treating or preventing restenosis in a mammal, comprising:

providing an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, said agent being a mimetic of P-selectin or the ligand; and

administering to a mammal an effective amount of said agent such that said P-selectin-ligand interaction is inhibited, wherein said agent is administered in conjunction with or after a vessel-corrective technique.--

Remarks

Pending Claims

Claims 1-38 have been canceled, and claims 39-63 have been added. Upon entry of this amendment, claims 39-63 will be pending. No new subject matter has been added.

Support for the newly added claims

Claims 39-50 and 63 are directed to methods of treating restenosis using an agent (e.g., an inhibitory protein or a glycoprotein) which inhibits a P-selectin-ligand interaction. Support for these claims can be found, e.g., starting at page 3, last paragraph through the first paragraph of page 4; at page 7, last paragraph; starting at page 8, second paragraph through page 10; at page 11, lines 3-16 and 27-28; at page 12, third paragraph; at page 13, second and fourth paragraphs; and at page 14, lines 20-25 of the specification.

Claims 51-50 are directed to methods of treating atherosclerosis using an agent (e.g., an inhibitory protein or a glycoprotein) which inhibits a P-selectin-ligand interaction. Support for these claims can be found, e.g., starting at page 3 through the first paragraph of page 4; at page 7, last paragraph; starting at page 8, second paragraph

through page 10; at page 11, lines 27-28; at page 12, third paragraph; at page 13, second and fourth paragraphs of the specification; and claims 1-38, as originally filed.

Claims 61-62 are directed to a chimeric construct for inhibiting an interaction between P-selectin and a ligand of P-selectin, comprising a P-selectin ligand or a fragment thereof (e.g., PSGL-1 or a fragment thereof) and another molecule. Support for these claims can be found, e.g., starting at page 3, last paragraph through page 4, first paragraph; and starting at page 8, last paragraph through page 9, first paragraph.

SUMMARY

If a telephone conversation with Applicant's Attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call Applicant's Attorney at (617) 542-5070.

Please charge any necessary fees to our Deposit Account No. 06-1050. The undersigned requests any extensions of time necessary to respond.

Respectfully submitted,
FISH & RICHARDSON PC

Louis Myers, Esq.
Reg. No. 35,965

225 Franklin Street
Boston, MA 02110
Tel. (617) 542-5070
Fax (617) 542-8906

Date 8/24/99

APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: **METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS**

APPLICANT: **DENISA D. WAGNER AND ROBERT C. JOHNSON**

"EXPRESS MAIL" Mailing Label Number EL278078485US

Date of Deposit November 8, 1999

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Joanne D. Boyle

Joanne D. Boyle

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METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS

This application is a continuation-in-part application of pending application Serial No. 08/253,663, filed on June 3, 1994, and entitled METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS. The entire contents of the parent application are hereby expressly incorporated by reference.

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant Nos. 7F32 HL08908 and P01 HL42443 awarded by the National Institutes of Health, and RO1 HL53756 awarded by the National Institutes of Health, National Heart, Lung and Blood Institute.

Field of the Invention

This invention relates to treatment and prevention of atherosclerosis.

Background of the Invention

Atherosclerosis is a principal cause of heart attacks, strokes and gangrene of the extremities. It has been reported that approximately 50% of all deaths in the United States, Europe and Japan are due to atherosclerosis. Atherosclerotic lesions can result from an excessive inflammatory-fibroproliferative response to various forms of insult to the endothelium and smooth muscle cells of the artery wall.

It is believed that the earliest type of atherosclerotic lesion is formed by binding of monocytes and T lymphocytes

($CD4^+$ and $CD8^+$) to the surfaces of endothelial cells in the lumen of the artery wall. These migrating cells proceed to penetrate beneath the arterial surface. The monocytes become macrophages, accumulate lipid, and become foam cells. These cells, together with the T lymphocytes, form a lesion called the fatty streak. The fatty streak subsequently develops into a fibrofatty intermediate lesion which is composed predominantly of layers of smooth muscle cells together with lipid-filled macrophages and T cells. These lesions in turn develop into complex occlusive lesions called fibrous plaques. The fibrous plaques can increase in size by projecting into the arterial lumen, and may thereby impede the flow of blood. Sudden death from myocardial infarctions can result from ruptures in the fibrous cap of the plaque, causing hemorrhage into the plaque, thrombosis and occlusion of the artery.

Current treatments for atherosclerosis include bypass grafting, endarterectomy, and angioplasty. These methods are high-risk invasive surgical procedures. Moreover, the failure rate of such treatments can often be high due to restenosis, which is thought to result from further inflammation, smooth muscle accumulation and thrombosis.

Summary of the Invention

It is an object of the invention to provide a safe, effective, easy and inexpensive method for treating or preventing atherosclerosis.

It is yet another object of the invention to provide a method for treating or preventing atherosclerosis which does not involve an invasive procedure.

It is yet another object of the invention to provide a simple method for treating or preventing atherosclerosis such as administering a pill, administering an injection or inserting an implant.

It is yet another object of the invention to treat or prevent atherosclerosis by administering to a mammal an agent which inhibits interaction between P-selectin and a ligand of P-selectin, so as to reduce formation of atherosclerotic lesions in the arteries.

Still another object of the invention is to provide a method for treating or preventing atherosclerosis by administering an agent which inhibits P-selectin function, and in which P-selectin function is restored upon depletion of the agent.

According to the invention, a method for treating or preventing atherosclerosis in a mammal is provided. An agent is provided for inhibiting interaction between P-selectin and a ligand of P-selectin. The agent is administered to a mammal in need of such treatment to cause this inhibition to occur.

In certain embodiments, the P-selectin is on a cell, preferably an endothelial cell or a platelet. The ligand preferably is a carbohydrate, e.g., sialyl-Lewis x, sialyl-Lewis a, sialyl-Lewis x-pentasaccharide, polylactosaminoglycan, a carbohydrate containing 2,6 sialic acid, Lewis x 3'-0-sulfate, or heparin oligosaccharides, or a glycoprotein, e.g., PSGL-1, 160 kD monospecific P-selectin ligand, or lysosomal membrane glycoproteins. The ligand can be on, e.g., monocytes, neutrophils, eosinophils, $CD4^+$ T cells, $CD8^+$ T cells or natural killer cells.

The agent can be, e.g., a soluble form of at least a portion of P-selectin or the ligand or mixtures thereof; an inhibitory protein, e.g., an antibody, e.g., a polyclonal or a monoclonal antibody, against at least a portion of P-selectin or the ligand or mixtures thereof; an inhibitory peptide, e.g., consisting of at least a portion of one of the binding sites on P-selectin or the ligand or mixtures thereof; an inhibitory carbohydrate, e.g., sialyl-Lewis x or

its analogs, sialyl-Lewis a or its analogs, heparin oligosacchardies or carbohydrates containing 2,6 sialic acids; an inhibitory glycoprotein, e.g., PSGL-1, 160 kD monospecific P-selectin ligand, lysosomal membrane glycoprotein or glycoprotein containing sialyl Lewis X; an inhibitory sulfatide; analogs of P-selectin or the ligand or mixtures thereof; substances derived from natural products, e.g., snake venoms or plant extracts; inhibitors of granular release; or inhibitors of a molecule required for the synthesis, post-translational modification, or functioning of P-selectin or the ligand.

In certain embodiments, the agent inhibits interaction between P-selectin and the ligand so as to at least partially prevent formation of, or to at least partially reverse a formed, atherosclerotic fatty streak, and/or an intermediate lesion, and/or a fibrous plaque, or so as to at least partially prevent growth of an atherosclerotic lesion after a surgical procedure for preventing restenosis.

Variations of this method of this invention include administering the agent prior to formation of an atherosclerotic lesion, administering the agent subsequent to formation of an atherosclerotic lesion, and administering the agent to a human.

Another aspect of the invention is a therapeutic agent in a dosage form and concentration suitable for treating or preventing atherosclerosis in a mammal in need of such treatment, the agent being effective to inhibit interaction between P-selectin and a ligand of P-selectin.

The above and other objects, features and advantages of the present invention will be better understood from the following specification.

Detailed Description

This invention provides a method for treating or preventing atherosclerosis in a mammal. An agent is provided which inhibits interaction between P-selectin and a ligand of P-selectin. The agent is administered to a mammal in need of such treatment to cause this inhibition to occur.

Atherosclerosis is a condition which is meant to include the presence of any one or more types of atherosclerotic lesions on the surface of an arterial wall. Such lesions include fatty streaks, fibrofatty intermediate lesions and fibrous plaques. Atherosclerosis develops in many mammals. By mammals is meant human as well as non-human mammals. Treating atherosclerosis is meant to include preventing, arresting, altering, and reversing formation of atherosclerotic lesions.

P-selectin is a cell surface adhesion receptor. A receptor is a transmembrane protein with three major domains. The extracellular domain has an active site on the exterior side of the membrane which recognizes and binds to a ligand. A short hydrophobic domain makes up the transmembrane portion, and an intracellular cytoplasmic domain transmits a signal to the cell that the ligand has bound to the receptor. The extracellular domain of P-selectin includes a Ca^{++}-dependent C-type lectin domain, an epidermal growth factor-like domain, and a series of consensus repeats related to those of complement-binding proteins.

P-selectin is expressed in various cells, including endothelial cells and platelets. P-selectin mediates adhesion of different types of cells to each other. For example, P-selectin typically mediates heterotypic interactions of platelets or endothelial cells with blood cells. Cells which bind to P-selectin include monocytes,

neutrophils, eosinophils, $CD4^+$ T cells, $CD8^+$ T cells and natural killer cells.

The binding of P-selectin to another cell can result from recognition of a ligand for P-selectin on that cell. By ligand is meant a moiety which binds to P-selectin, the moiety being either alone or attached to another molecule. P-selectin ligands include carbohydrate groups, e.g., sialyl-Lewis X (Foxall et al., J. Cell Biol., 117(4): 895-902, 1992; Polley et al., Proc. Nat'l Acad. Sci., USA, 88:6224-6228, 1991) sialyl-Lewis a (Berg et al., J. Biol. Chem., 266: 14869-14875, 1991), sialyl-Lewis x pentasaccharide (Mulligan et al., Nature 364: 149-151, 1993), polylactosaminoglycan, carbohydrate containing 2,6 sialic acid (Larsen et al., J. Biol. Chem. 267: 11104-11110, 1992), Lewis x 3'-0-sulfate (Yuen et al., Biochemistry 31: 9126-9133, 1992) and heparin oligosaccharides (Nelson et al., Blood 82: 3253-3258, 1993). P-selectin ligands are also meant to include glycoproteins which contain a carbohydrate structure. For example, a P-selectin carbohydrate ligand can be linked to a mucin-like molecule. (Sako et al., Cell 75(6): 1179-1186, 1993; Linter et al., J. Biol. Chem. 125: 471-481, 1994). By mucin is meant serine- and threonine-rich proteins that are heavily O-glycosylated and have an extended structure. Other glycoprotein ligands include PSGL-1, 160 kD monospecific P-selectin ligand (Linter et al., J. Biol. Chem. 125: 471-481, 1994) and lysosomal membrane glycoproteins (Fukuda, J. Biol. Chem. 266: 21327-21332, 1991). Analogs of the above ligands which can bind to P-selectin, e.g., where fucose is replaced, e.g., by a diol group, or derivatives of the sialyl-Lewis x compounds which carry a SO_3^- group instead of sialic acid, or contain a sialic acid in a 2,6 linkage, are also meant to be included as P-selectin ligands.

It is known that P-selectin is involved in cellular responses to inflammation resulting from injury or

infection. This invention demonstrates that P-selectin can also be involved in the formation of atherosclerotic lesions. Example 1 shows that the presence in mice of a homozygous null mutation in P-selectin significantly decreases the size of the atherosclerotic lesions that are formed when the mice are fed a high fat diet, as compared to wild-type mice fed a high fat diet. The general health of these homozygous P-selectin deficient mice appear normal up to at least two years of age. The fact that these P-selectin deficient mice are viable, fertile, of normal size and vigor, and free of obvious signs of infection or disease, demonstrates that P-selectin is not required for normal development. The mouse fed a high fat diet, or various genetically engineered mice, are generally accepted as a good model for atherosclerosis in humans. (Lusis, Trends in Cardiovascular Medicine, 3: 135-143, 1993; Stoltzfus and Rubin, Trends in Cardiovascular Medicine, 3: 130-134, 1993; Ishida and Paigen, In Genetic Factors in Atherosclerosis; Monogr. Hum. Gen., Vol. 12: 189-222, 1989). Significantly, Example 4 shows that the presence in mice of a homozygous null P-selectin mutation also causes a significant reduction in the size of atherosclerotic lesions that are formed when the mice have in addition an LDL receptor-deficient mutation. Mice which lack LDL are a model system for the human disease called homozygous familial hypercholesterolemia (see Ishibashi et al., J. Clin. Invest., 93:1885-1893 (1994)), in which functional LDL receptor is absent, and as a consequence cholesterol-rich lipoproteins accumulate in the plasma, resulting in atherosclerotic lesions in childhood.

The agent of this invention can inhibit interaction between P-selectin and a ligand of P-selectin. By inhibiting interaction is meant, e.g., that P-selectin and its ligand are unable to properly bind to each other to effect proper formation of atherosclerotic lesions. Such inhibition can be

the result of any one of a variety of events, including, e.g., preventing or reducing interaction between P-selectin and the ligand, inactivating P-selectin and/or the ligand, e.g., by cleavage or other modification, altering the affinity of P-selectin and the ligand for each other, diluting out P-selectin and/or the ligand, preventing surface, plasma membrane, expression of P-selectin or reducing synthesis of P-selectin and/or the ligand, synthesizing an abnormal P-selectin and/or ligand, synthesizing an alternatively spliced P-selectin and/or ligand, preventing or reducing proper conformational folding of P-selectin and/or the ligand, modulating the binding properties of P-selectin and/or the ligand, interfering with signals that are required to activate or deactivate P-selectin and/or the ligand, activating or deactivating P-selectin and/or the ligand at the wrong time, or interfering with other receptors, ligands or other molecules which are required for the normal synthesis or functioning of P-selectin and/or its ligand.

Examples of agents include soluble forms of P-selectin or the ligand, inhibitory proteins, inhibitory peptides, inhibitory carbohydrates, inhibitory glycoproteins, inhibitory glycopeptides, inhibitory sulfatides, synthetic analogs of P-selectin or the ligand, certain substances derived from natural products, inhibitors of granular release, and inhibitors of a molecule required for the synthesis or functioning of P-selectin or the ligand.

The soluble form of either P-selectin or the ligand, or a portion thereof, can compete with its cognate molecule for the binding site on the complementary molecule, and thereby reduce or eliminate binding between the membrane-bound P-selectin and the cellular ligand. The soluble form can be obtained, e.g., from purification or secretion of naturally occurring P-selectin or ligand, from recombinant P-selectin

or ligand, or from synthesized P-selectin or ligand. Soluble forms of P-selectin or ligand are also meant to include, e.g., truncated soluble secreted forms, proteolytic fragments, other fragments, and chimeric constructs between at least a portion of P-selectin or ligand and other molecules. Soluble forms of P-selectin are described in Mulligan et al., J. Immunol., 151: 6410-6417, 1993, and soluble forms of P-selectin ligand are described in Sako et al., Cell 75(6): 1179-1186, 1993.

Inhibitory proteins include, e.g., anti-P-selectin antibodies (Palabrica et al., Nature 359: 848-851, 1992; Mulligan et al., J. Clin. Invest. 90: 1600-1607, 1992; Weyrich et al., J. Clin. Invest. 91: 2620-2629, 1993; Winn et al., J. Clin. Invest. 92: 2042-2047, 1993); anti-P-selectin ligand antibodies (Sako et al., Cell 75(6): 1179-1186, 1993); Fab_2 fragments of the inhibitory antibody generated through enzymatic cleavage (Palabrica et al., Nature 359: 848-851, 1992); P-selectin-IgG chimeras (Mulligan et al., Immunol. 151: 6410-6417, 1993); and carrier proteins expressing a carbohydrate moiety recognized by P-selectin. The antibodies can be directed against P-selectin or the ligand, or a subunit or fragment thereof. Both polyclonal and monoclonal antibodies can be used in this invention. Preferably, monoclonal antibodies are used. Most preferably, the antibodies have a constant region derived from a human antibody and a variable region derived from an inhibitory mouse monoclonal antibody. Antibodies to human P-selectin are described in Palabrica et al., Nature 359: 848-851, 1992; Stone and Wagner, J.C.I., 92: 804-813, 1993; and to mouse P-selectin are described in Mayadas et al., Cell, 74: 541-554, 1993. Antibodies to human ligand are described in Sako et al., Cell 75(6): 1179-1186, 1993. Antibodies that are commercially available against human P-selectin include clone AC1.2 monoclonal from Becton Dickinson, San Jose, CA.

An inhibitory peptide can, e.g., bind to a binding site on the P-selectin ligand so that interaction as by binding of P-selectin to the ligand is reduced or eliminated. The inhibitory peptide can be, e.g., the same, or a portion of, the primary binding site of P-selectin, (Geng et al., J. Biol. Chem., 266: 22313-22318, 1991, or it can be from a different binding site. Inhibitory peptides include, e.g., peptides or fragments thereof which normally bind to P-selectin ligand, synthetic peptides and recombinant peptides. In another embodiment, an inhibitory peptide can bind to a molecule other than P-selectin or its ligand, and thereby interfere with the binding of P-selectin to its ligand because the molecule is either directly or indirectly involved in effecting the synthesis and/or functioning of P-selectin and/or its ligand.

Inhibitory carbohydrates include oligosaccharides containing sialyl-Lewis a or sialyl-Lewis x or related structures or analogs, carbohydrates containing 2,6 sialic acid, heparin fractions depleted of anti-coagulant activity, heparin oligosaccharides, e.g., heparin tetrasaccharides or low weight heparin, and other sulfated polysaccharides. Inhibitory carbohydrates are described in Nelson et al., Blood 82: 3253-3258, 1993; Mulligan et al., Nature 364: 149-151, 1993; Ball et al., J. Am. Chem. Soc. 114: 5449-5451, 1992; De Frees et al., J. Am. Chem. Soc. 115: 7549-7550, 1993. Inhibitory carbohydrates that are commercially available include, e.g., 3'-sialyl-Lewis x, 3'-sialyl-Lewis a, lacto-N-fucopentose III and 3'-sialyl-3-fucosyllactose, from Oxford GlycoSystems, Rosedale, NY.

Inhibitory glycoproteins, e.g., PSGL-1, 160 kD monospecific P-selectin ligand, lysosomal membrane glycoproteins, glycoprotein containing sialyl-Lewis x, and inhibitory sulfatides (Suzuki et al., Biochem. Biophys. Res. Commun. 190: 426-434, 1993; Todderud et al., J. Leuk. Biol.

52: 85-88, 1992) that inhibit P-selectin interaction with its ligand can also be used in this invention.

Synthetic analogs or mimetics of P-selectin or the ligand also can serve as agents. P-selectin analogs or mimetics are substances which resemble in shape and/or charge distribution P-selectin. An analog of at least a portion of P-selectin can compete with its cognate membrane-bound P-selectin for the binding site on the ligand, and thereby reduce or eliminate binding between the membrane-bound P-selectin and the ligand. Ligand analogs or mimetics include substances which resemble in shape and/or charge distribution the carbohydrate ligand for P-selectin. An analog of at least a portion of the ligand can compete with its cognate cellular ligand for the binding site on the P-selectin, and thereby reduce or eliminate binding between P-selectin and the cellular ligand. In certain embodiments which use a ligand analog, the sialic acid of a carbohydrate ligand is replaced with a group that increases the stability of the compound yet still retains or increases its affinity for P-selectin, e.g. a carboxyl group with an appropriate spacer. An advantage of increasing the stability is that it allows the agent to be administered orally. Sialyl-Lewis x analog with glucal in the reducing end and a bivalent sialyl-Lewis x anchored on a galactose residue via ß-1,3- and ß-1,6- linkages also inhibit P-selectin binding (DeFrees et al., J. Am. Chem. Soc., 115: 7549-7550, 1993).

Agents are also meant to include substances derived from natural products, such as snake venoms and plant extracts, that inhibit P-selectin interaction with its ligand. Such substances can inhibit this interaction directly or indirectly, e.g., through specific proteolytic cleavage or other modification of P-selectin or its ligand.

An inhibitor of granular release also interferes with P-selectin expression on the cell surface, and therefore

interferes with P-selectin function. By granular release is meant the secretion by exocytosis of storage granules containing P-selectin: Weibel-Palade bodies of endothelial cells or α-granules of platelets. The fusion of the granular membrane with the plasma membrane results in expression of P-selectin on the cell surface. Examples of such agents include colchicine. (Sinha and Wagner, Europ. J. Cell. Biol. 43: 377-383, 1987).

Agents also include inhibitors of a molecule that is required for synthesis, post-translational modification, or functioning of P-selectin and/or the ligand. Agents include cytokines, growth factors, hormones, signaling components, kinases, phosphatases, homeobox proteins, transcription factors, translation factors and post-translation factors or enzymes. Agents are also meant to include ionizing radiation, non-ionizing radiation, ultrasound and toxic agents which can, e.g., at least partially inactivate or destroy P-selectin and/or the ligand.

An agent is also meant to include inhibitors which are not entirely P-selectin specific. For example, an agent may inhibit other selectin interactions in addition to P-selectin interactions, e.g., L and/or E selectin interactions. Such overlapping specificity may provide additional therapeutic advantage.

Administration of the agent can be accomplished by any method which allows the agent to reach the target cells. These methods include, e.g., injection, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the agent is obtained. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g.,

microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the agent with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

Administration of the agent can be alone or in combination with other therapeutic agents. In certain embodiments, the agent can be combined with a suitable carrier, incorporated into a liposome, or incorporated into a polymer release system.

Preferably, protein agents are administered by intravenous or intramuscular injection; peptide agents by intravenous or intramuscular injection or by glycerin suppository; carbohydrate or sulfatide agents by intravenous or intramuscular injection, or with an aerosol in an inhalator; and synthetic analog agents by intravenous or intramuscular injection, or with an aerosol in an inhalator, or orally.

In certain embodiments of the invention, the administration can be designed so as to result in sequential exposures to the agent over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the agent by one of the methods described above, or alternatively, by a controlled release delivery system in which the agent is delivered to the mammal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the agent does not occur immediately upon administration, but rather is delayed for some time period. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g.,

by long acting oral dosage forms, bolus injections, transdermal patches and sub-cutaneous implants.

Examples of systems in which release occurs in bursts include, e.g., systems in which the agent is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimuli, e.g., temperature, pH, light or a degrading enzyme, and systems in which the agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the agent is gradual and continuous include, e.g., erosional systems in which the agent is contained in a form within a matrix, and diffusional systems in which the agent permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

The agent can be suspended in a liquid, e.g., in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases water or an organic liquid can be used.

The agent can be administered prior to or subsequent to fibrous plaque formation. In certain embodiments, the agent is administered to patients, e.g., after angioplasty, stenting procedure, atherectomy, or bypass surgery or other vessel-corrective techniques, to aid in preventing restenosis. The agent also can be administered, preferably on a daily basis, to patients with familial hypercholesteremia, an early debilitating disease, who develop atherosclerotic lesions at a young age, often resulting in arterial narrowing and death.

The agent is administered to the mammal in a therapeutically effective amount. By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing plaque formation. A therapeutically effective amount can be determined on an

individual basis and will be based, at least in part, on consideration of the species of mammal, the mammal's size, the agent used, the type of delivery system used, the time of administration relative to plaque formation, and whether a single, multiple, or controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Preferably, the concentration of an inhibitory protein, peptide, glycoprotein or glycopeptide if applied systemically, is at a dose of about 0.1 to about 500 mg/kg body weight. Most preferably the dose is about 0.1 to about 5 mg/kg. The specific concentration partially depends upon the particular inhibitory protein, glycoprotein, peptide or glycopeptide used, as some are more effective than others. Preferably, the concentration of a carbohydrate or a synthetic analog, if applied systemically is at a dose of about 0.01 to about 200 mg/kg body weight. Most preferably, the dose is about 0.1 to about 5 mg/kg. Preferably, the concentration of a sulfatide, if applied systemmically is at a dose of about 1 to about 100 mg/kg body weight. Preferably, the concentration of a soluble form of P-selectin or ligand, if applied systemically is at a dose of about 1 to about 100 mg/kg body weight. Most preferably, the dose is about 1 to about 5 mg/kg. The dosage concentration of the agent that is actually administered is dependent at least in part upon the final concentration that is desired at the site of action, the method of administration, the efficacy of the particular agent, the longevity of the particular agent, and the timing of administration relative to the formation of the atherosclerotic lesion. Preferably, the dosage form is such that it does not substantially deleteriously affect the mammal. The dosage can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

The agents of the invention are meant to include reversible and non-reversible agents. If an agent is reversible, the inhibition of the interaction between P-selectin and its ligand will be reversed at some point after administration of the agent ceases. A reversible agent is preferable in that it permits discontinuation of administration of the agent during periods of infection or wounds. P-selectin function is thereby restored and able to act in its inflammation-response capacity to aid in fighting infections or in wound repair.

The invention also includes a therapeutic agent in a dosage form and concentration suitable for treating or preventing atherosclerosis in a mammal in need of such treatment, the agent being effective to inhibit interaction between P-selectin and its ligand.

EXAMPLES

Example 1: P-Selectin-Deficient Mice Fed a High Fat Diet Have Significantly Smaller Atherosclerotic Lesions Than Wild-Type Mice

This example illustrates that P-selectin plays an important role in the formation of atherosclerotic lesions in blood vessels. Comparisons were made of atherosclerotic lesions in wild-type and P-selectin-deficient mice fed a high fat diet. The P-selectin deficient mice contain a homozygous null mutation in P-selectin and were generated by homologous recombination in embryonic stem cells as described in Mayadas et al., Cell 74: 541-554, 1993.

Age-matched female wild-type and P-selectin deficient mice were used. (C57BL and 129 mixed background; both of these strains are susceptible to aortic lesion formation upon $\geq$ 14 week exposure to a high fat diet.). The mice were

anesthetized and bled from the retroorbital venous plexus at the initiation of the prescribed diets. They were divided into two groups, each consisting of wild-type and P-selectin deficient mice. The control low fat group was fed Purina mouse chow containing 4.5% (w/w) animal fat, 0.03% (w/w) cholesterol, no sodium cholate and no casein. The other group was fed a high fat diet containing 15% (w/w) fat (from butter), 1.15-1.25% (w/w) cholesterol, 0.5% (w/w) sodium cholate and 20% casein (Rubin et al., Nature 353: 265-267 (1991)). The mice were started on the diets at 12-16 weeks of age and maintained on the diets for 19-21 weeks, at which time blood was drawn and the mice were sacrificed.

The total cholesterol levels in the blood plasma increased by comparable amounts in both P-selectin-deficient and wild-type mice. The p value is 0.46, indicating that there was no statistical difference in cholesterol levels in response to the high fat diet in the two sets of mice. The measured cholesterol value increases were similar to those reported by Paigen et al., Atherosclerosis, 57: 65-75, 1985.

The hearts were processed according to Paigen et al., Atherosclerosis, 68: 231-240 (1987). The heart and attached aorta were placed in 0.9% saline for 1 hour to remove erythrocytes and allow muscle relaxation. The hearts were then fixed in 10% buffered formalin and embedded in gelatin. For quantitative evaluation, the hearts were embedded in O.C.T. (optimal cooling temperature) compound, frozen and sectioned on a cryostat. Sections were discarded until reaching the junction of the heart muscle and aorta where the valve cusps become visible and the aorta is rounded. Unstained sections were regularly examined to locate the area of interest. This area of the aorta was shown previously to consistently result in lesions in C57BL/6 mice following 14 weeks exposure to the high fat diet. (Paigen et al., Atherosclerosis, 68: 231-240, 1987). Once the area was

localized, four consecutive 10 μm sections were collected for each slide. Sectioning continued for approximately 350 μm (9-10 slides/heart) towards the aortic arch and exiting the valve region. Sections were collected onto gelatin coated glass slides and odd numbered slides were stained with oil red-O and hematoxylin. Tissues were then counterstained with light green.

One section on each of the odd numbered slides was assessed. Where possible, the same section on each of the five slides was used for quantitation. Thus, five sections, each 80 μm apart, were examined. If a section on a slide was folded or damaged, then the section immediately following or preceding replaced the flawed section. The slides were coded and the examiner was unaware of the genotype of the animal from which the sections originated. The size of the lesion was quantified using an ocular micrometer (net grid with 100 squares; each square 25 x 25 μm using 40x objective). Lesions less than 0.1 square using the 40x objective (400x magnification) were not counted. Lesions for each section were totaled. As shown in Table 1, the average size of the lesions in the P-selectin deficient mice fed a high fat diet was 3.6 times smaller than for the wild-type mice fed a high fat diet. No aortic lesions were present in wild-type or P-selectin deficient mice (one each) fed the low fat control diet.

TABLE 1

SIZE (μm^2) OF ATHEROSCLEROTIC LESIONS IN WILD TYPE AND P-SELECTIN-DEFICIENT MICE

(five values per mouse, each 80 microns apart)

Wild Type	P-Selectin-Deficient
562.50	406.25
1375.00	1000.00
2000.00	562.50
562.50	687.50
1062.50	937.50
4750.00	0.00
2937.50	312.50
13000.00	1250.00
1375.00	187.50
0.00	750.00
0.00	62.50
2250.00	125.00
2250.00	437.50
2937.50	1125.00
0.00	750.00
1250.00	218.75
375.00	0.00
437.50	187.50
625.00	0.00
250.00	0.00
437.50	0.00
250.00	187.50
187.50	93.75
125.00	187.50
125.00	218.75
0.00	500.00
0.00	625.00
0.00	9625.00

TABLE 1 continued:

Wild Type	P-Selectin-Deficient
0.00	2812.50
0.00	437.50
812.50	0.00
875.00	187.50
4875.00	125.00
3812.50	187.50
4437.50	562.50
0.00	0.00
0.00	0.00
62.50	0.00
187.50	0.00
1312.50	0.00
5875.00	
2625.00	
6000.00	
6812.50	
7875.00	
4750.00	
5937.50	
5687.50	
9125.00	
437.50	

Statistical comparison of the lesion formation in the wild-type and P-selectin-deficient mice was done using the student t-test. Each mouse provided five individual values for statistical evaluation. Other investigators have previously determined that lesions 80 μm equidistant apart are likely to represent separate events and can therefore be computed separately. (Paigen et al, Atherosclerosis, 68: 231-240, 1987).

As Table 2 demonstrates, analysis of the atherosclerotic lesion data shows that the obtained t-statistic could have occurred by chance two times out of a thousand, and therefore the difference in the size of the lesions in the wild-type and P-selectin-deficient mice are highly statistically different.

TABLE 2

t-TEST: TWO-SAMPLE ASSUMING EQUAL VARIANCES

	Wild Type	P-Selectin-Deficient
Mean	2,212.50	618.75
Variance	8,306,760.20	2,405,408.65
Observations	50.00	40.00
Pooled Variance	5,691,388.49	
Hypothesized Mean Difference	0	
df	88	
t Stat	3.149	
P(T<=t) two-tail	0.002	

Example 2: Treating Atherosclerosis in a Human with Sialyl Lewis x

This example illustrates a method for treating atherosclerosis in a human with an agent which inhibits interaction between P-selectin and its ligand. The patient is given an intramuscular injection of sialyl-Lewis x, once a day for a period of six months. (Mulligan et al., Nature 364: 149-151, 1993). The dose concentration per day is 1 mg/kg body weight. This treatment interferes with further development of atherosclerotic lesions.

Example 3: Treating Atherosclerosis in a Human With an Analog of Sialyl-Lewis x

This example illustrates a method for treating atherosclerosis in a human with an agent which inhibits

interaction between P-selectin and its ligand. The patient is given a synthetic analog of a carbohydrate ligand orally, in the form of a pill, once a day. The compound is a mimetic of a carbohydrate ligand for P-selectin similar in size and charge distribution to sialyl-Lewis x. The analog is synthesized using both enzymatic (use of highly purified glycosyltransferases and glycosidases) and conventional chemical methods. The compound has a rigid structure to fix its conformation to that of the highest affinity for P-selectin. The original position of the sialic acid is occupied by a carboxyl group, and that of the fucose by a hydrogen donor, a triol. The dose concentration per day is 1 mg/kg body weight. Administration is carried out for a period of three months. This treatment interferes with further development of atherosclerotic lesions.

Example 4: Mice Lacking LDL Receptor, a Mouse Model for Human Homozygous Familial Hypercholesterolemia, Develop Significantly Smaller Atherosclerotic Lesions If They Are Also Deficient in P-Selectin

This example illustrates that the absence of P-selectin can significantly attenuate the severe phenotype of heart disease in mice lacking LDL receptor -- a situation genetically identical to a human disease called homozygous familial hypercholesterolemia (FH). In humans with FH, the absence of functional LDL receptor leads to the accumulation of cholesterol-rich lipoproteins in plasma. As a consequence, macrophages loaded with cholesteryl esters are deposited throughout the body and atherosclerotic lesions of the aortic root and coronary arteries develop in childhood. (See Goldstein and Brown, Familial Hypercholesterolemia. In The Metabolic Basis of Inherited Disease, eds. Scriver et al., McGraw Hill Inc., N.Y. 1215-1250 (1989)).

To examine whether the absence of P-selectin can influence the development of the extensive atherosclerotic lesions in FH, the P-selectin-deficient mice described in Example 1 (Mayadas et al., Cell 74:541-554 (1993)) were bred with LDL receptor-deficient mice developed through gene targeting technology described in Ishibashi et al., J. Clin. Invest., 92:883-893 (1993). The phenotype of the LDL receptor-deficient mice is remarkably similar to the phenotype of human homozygous FH when the animals are fed an atherogenic diet rich in cholesterol, saturated fat, and cholic acid (Ishibashi et al., J. Clin. Invest., 93:1885-1893 (1994)). Through the above-described breeding, a colony of mice deficient for LDL receptor and either wild-type for P-selectin or homozygous-deficient for P-selectin have been obtained. Twelve mice which are LDL receptor-deficient and wild-type for P-selectin (P-selectin-positive), and 11 mice deficient for both LDL receptor and P-selectin (P-selectin-negative), were put on an atherogenic diet for 8 weeks. Their hearts were then processed as described in Example 1. Within two weeks of the onset of the diet, their plasma cholesterol reached levels above 1,000 mg/dl, as compared to 200 mg/dl prior to the diet administration. At the time of sacrifice, a large sample of blood was collected for individual cholesterol, triglyceride and lipoprotein profile analysis. No differences were detected between the P-selectin-negative and P-selectin-positive mice. After 8 weeks on the high cholesterol diet, the mice had practically no HDL, and most of the cholesterol was in the LDL-VLDL region, in agreement with results reported by others (Isibashi et al., J. Clin. Invest., 93:1885-1893 (1994)). Importantly, there was no difference in the total plasma cholesterol levels between the P-selectin-positive and negative mice -- both groups gave approximately 1000 mg/dl (levels comparable to those seen in human FH) (Table 3).

TABLE 3

CHOLESTEROL LEVELS IN LDLR-DEFICIENT MICE AFTER 8 WEEKS ON HIGH-FAT DIET (mg/dl)

P-Selectin Wild Type	P-Selectin-Deficient
842	1068
1066	1053
1088	1228
1021	1076
940	1176
1241	1025
1135	795
1046	1114
926	1036
1842	1024
1438	1283
1280	

Statistics:

	P-Selectin Wild Type	P-Selectin Deficient
mean	1155.42	1079.82
standard deviation	272.09	127.94
n	12	11
p value	0.411	

The results shown in Table 3 confirmed that the diet had the desired effect on plasma cholesterol level and also that the mice were correctly genotyped as LDL receptor-deficient. As described in Example 1, five sections of the aorta in the cusps regions were assessed. The mean area of the lesion was determined and this single value for each animal (Table 4) was used for statistical analysis (Tables 5 and 6).

TABLE 4

MEAN ATHEROSCLEROTIC LESION SIZE (mm^2)
IN LDL RECEPTOR-DEFICIENT MICE

(one value per mouse which is the mean from 5 sections, 80 microns apart)

P-Selectin Positive (total)	P-Selectin Negative (total)
0.267	0.082
0.149	0.189
0.283	0.207
*0.256	0.241
0.276	0.154
0.182	*0.240
0.253	*0.079
*0.436	*0.157
*0.279	*0.209
*0.177	0.100
0.097	*0.044
*0.336	

* indiates males

TABLE 5

t-TEST FOR P-SELECTIN-POSITIVE MICE
AND P-SELECTIN-NEGATIVE MICE:
TWO-SAMPLE ASSUMING EQUAL VARIANCES

	P-Selectin Positive (total)	P-Selectin Negative (total)
Mean	0.249	0.155
Variance	0.008	0.005
Observations	12.000	11.000
Pooled Variance	0.006	
Hypothesized Mean Difference	0.000	
df	21.000	
t Stat	2.810	
P(T<=t) two-tail	0.010	

TABLE 6

t-TEST FOR P-SELECTIN-POSITIVE MALE MICE AND P-SELECTIN-NEGATIVE MALE MICE: TWO-SAMPLE ASSUMING EQUAL VARIANCES

	P-Selectin Positive (males)	P-Selectin Negative (males)
Mean	0.296	0.146
Variance	0.009	0.007
Observations	5.000	5.000
Pooled Variance	0.008	
Hypothesized Mean Difference	0.000	
df	8.000	
t Stat	2.632	
P(T<=t) two-tail	0.030	

As shown in Table 4, the mean size of the atherosclerotic lesions in the P-selectin-positive mice was very large. Despite the overwhelming size of the atherosclerotic lesions in this FH model, the absence of P-selectin caused a significant reduction in lesion size (Table 5). This result was especially notable in males, where the lesions in the P-selectin-positive mice were twice the size of those found in P-selectin-negative animals (Table 6).

Those skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

What is claimed is:

CLAIMS

1. A method for treating or preventing atherosclerosis in a mammal, comprising:

providing an agent for inhibiting interaction between P-selectin and a ligand of P-selectin, and

administering said agent to a mammal in need of such treatment to cause such inhibition to occur.

2. The method of claim 1 wherein said P-selectin is on a cell.

3. The method of claim 2 wherein said cell is an endothelial cell.

4. The method of claim 2 wherein said cell is a platelet.

5. The method of claim 1 wherein said ligand comprises a carbohydrate.

6. The method of claim 1 wherein said ligand comprises a glycoprotein.

7. The method of claim 1 wherein said ligand is selected from the group consisting of sialyl-Lewis x, sialyl-Lewis a, sialyl-Lewis x-pentasaccharide, polylactosaminoglycan, carbohydrate containing 2,6 sialic acid, Lewis x 3'-0-sulfate, heparin oligosaccharides, PSGL-1, 160 kD monospecific P-selectin ligand and lysosomal membrane glycoproteins.

8. The method of claim 1 wherein said ligand is on a cell selected from the group consisting of monocytes, neutrophils, eosinophils, $CD4^+$ T cells, $CD8^+$ T cells, and natural killer cells.

9. The method of claim 1 wherein said ligand is on a leukocyte.

10. The method of claim 9 wherein said leukocyte is a neutrophil.

11. The method of claim 9 wherein said leukocyte is a monocyte.

12. The method of claim 1 wherein said P-selectin can bind to said ligand in the absence of said agent.

13. The method of claim 1 wherein said agent is selected from the group consisting of a soluble form of at least a portion of said P-selectin and a soluble form of at least a portion of said ligand and mixtures thereof.

14. The method of claim 1 wherein said agent is an inhibitory protein.

15. The method of claim 14 wherein said inhibitory protein is selected from the group consisting of an antibody against at least a portion of said P-selectin and an antibody against at least a portion of said ligand and mixtures thereof.

16. The method of claim 15 wherein said antibody is a monoclonal antibody.

17. The method of claim 1 wherein said agent is an inhibitory peptide.

18. The method of claim 17 wherein said P-selectin has a first binding site for said ligand and said ligand has a second binding site for said P-selectin, and wherein said inhibitory peptide is a peptide selected from the group consisting of at least a portion of said first binding site and at least a portion of said second binding site and mixtures thereof.

19. The method of claim 1 wherein said agent is an inhibitory carbohydrate.

20. The method of claim 19 wherein said inhibitory carbohydrate is selected from the group consisting of sialyl-Lewis x and its analogs, sialyl Lewis a and its analogs, heparin oligosaccharides and carbohydrates containing 2,6 sialic acid.

21. The method of claim 1 wherein said agent is an inhibitory glycoprotein.

22. The method of claim 21 wherein said inhibitory glycoprotein is selected from the group consisting of PSGL-1, 160 kD monospecific P-selectin ligand, lysosomal membrane glycoprotein and glycoprotein containing sialyl-Lewis x.

23. The method of claim 1 wherein said agent is an inhibitory sulfatide.

24. The method of claim 1 wherein said agent is selected from the group consisting of an analog of said P-selectin and an analog of said ligand and mixtures thereof.

25. The method of claim 1 wherein said agent is a substance derived from snake venom or a plant extract.

26. The method of claim 1 wherein said agent is an inhibitor of granular release.

27. The method of claim 1 wherein said agent is an inhibitor of a molecule required for the synthesis, post-translational modification or functioning of said P-selectin or said ligand.

28. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially prevent formation of an atherosclerotic fatty streak.

29. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially prevent formation of an atherosclerotic intermediate lesion.

30. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially prevent formation of an atherosclerotic fibrous plaque.

31. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially prevent growth of an atherosclerotic lesion after a surgical procedure for at least partially preventing restenosis.

32. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially reverse a formed atherosclerotic fatty streak.

33. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially reverse a formed atherosclerotic intermediate lesion.

34. The method of claim 1 wherein said agent inhibits interaction between said P-selectin and said ligand so as to at least partially reverse a formed atherosclerotic fibrous plaque.

35. The method of claim 1 wherein said administering occurs prior to formation of an atherosclerotic lesion.

36. The method of claim 1 wherein said administering occurs subsequent to formation of an atherosclerotic lesion.

37. The method of claim 1 wherein said mammal is a human.

38. A therapeutic agent in a dosage form and concentration suitable for treating or preventing atherosclerosis in a mammal in need of such treatment, said agent being effective to inhibit interaction between P-selectin and a ligand of P-selectin.

METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS

ABSTRACT

A method for treating or preventing atherosclerosis in a mammal is described. An agent for inhibiting interaction between P-selectin and a ligand of P-selectin is provided. The agent is administered to a mammal in need of such treatment to cause this inhibition to occur.

1302g/7-8
(REV. 03-24-93)

Docket No. C0279/7017

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS

the specification of which

(check one)

☐ is attached hereto

☒ was filed on January 24, 1995, as Application Ser. No. 08/377,798.

☐ was filed as a PCT international application No. , on and was amended under PCT Article 19 on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign PCT Application(s) and any priority claims under 35 U.S.C. §119:			Priority Claimed	
(Number)	(Country if PCT so indicate)	(Day/Month/Year Filed)	YES	NO
(Number)	(Country)	(Day/Month/Year Filed)	YES	NO
(Number)	(Country)	(Day/Month/Year Filed)	YES	NO

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

08/253,663	June 3, 1994	pending
(Application Serial No.)	(filing date)	(status-patented, pending, abandoned)
(Application Serial No.)	(filing date)	(status-patented, pending, abandoned)

PCT Applications designating the United States:

(PCT Application No.)	(U.S. Ser. No.)	(PCT filing date)	(status-patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

David Wolf	17,528	James J. Foster	30,052	Douglas R. Wolf	36,971
George L. Greenfield	17,756	Charles E. Pfund	17,030	Helen A. Greer	36,816
Stanley Sacks	19,900	Philip G. Koenig	30,186	Elizabeth R. Plumer	36,637
David M. Driscoll	25,075	Anthony J. Mirabito	28,161	Timothy J. Oyer	36,628
Edward F. Perlman	28,105	Jason M. Honeyman	31,624	Kristofer E. Elbing	34,590
Lawrence M. Green	29,384	William A. Loginov	34,863	John N. Anastasi	37,765
Steven J. Henry	27,900	James H. Morris	34,681	Brett N. Dorny	35,860
Therese A. Hendricks	30,389	Anthony J. Janiuk	29,809	David L. Cavanaugh	36,476
Edward R. Gates	31,616	Peter C. Lando	34,654	Michael J. Twomey	38,349
William R. McClellan	29,409	Gary S. Engelson	35,128	Helen C. Kindregan	P39,248
Ronald J. Kransdorf	20,004	Peter J. Gordon	35,164	James M. Hanifin, Jr.	P39,213
M. Lawrence Oliverio	30,915	Randy J. Pritzker	35,986	David E. Huang	P39,229
		Richard F. Giunta	36,149	Bruce D. Rubenstein	P39,349
				Thomas M. Sullivan	P39,392

Address all telephone calls to Helen Greer at telephone no. (617) 720-3500. Address all correspondence to Helen Greer

c/o Wolf, Greenfield & Sacks, P.C.
Federal Reserve Plaza
600 Atlantic Avenue
Boston, MA 02210-2211

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Inventor's signature Denisa D. Wagner Date 3/17/95
Full name of sole or first inventor Denisa D. Wagner
Citizenship U.S.A.
Residence 21 Richland Road, Wellesley, MA 02181
Post Office Address SAME AS ABOVE

Inventor's signature Robert C. Johnson Date 3/17/95
Full name of joint inventor Robert C. Johnson
Citizenship U.S.A.
Residence 55 Fox Trail Road, Sparta, NJ 07871
Post Office Address SAME AS ABOVE

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